Metronomic chemotherapy: new rationale for new directions

Eddy Pasquier, Maria Kavallaris and Nicolas André

Abstract | Tumor angiogenesis is recognized as a major therapeutic target in the fight against cancer. The key involvement of angiogenesis in tumor growth and metastasis has started to redefine chemotherapy and new protocols have emerged. Metronomic chemotherapy, which is intended to prevent tumor angiogenesis, is based on more frequent and low-dose drug administrations compared with conventional chemotherapy. The potential of metronomic chemotherapy was revealed in animal models a decade ago and the efficacy of this approach has been confirmed in the clinic. In the past 5 years, multiple clinical trials have investigated the safety and efficacy of metronomic chemotherapy in a variety of human cancers. While the results have been variable, clinical studies have shown that these new treatment protocols represent an interesting alternative for either primary systemic therapy or maintenance therapy. We review the latest clinical trials of metronomic chemotherapy in adult and pediatric cancer patients. Accumulating evidence suggests that the efficacy of such treatment may not only rely on anti-angiogenic activity. Potential new mechanisms of action, such as restoration of anticancer immune response and induction of tumor dormancy are discussed. Finally, we highlight the research efforts that need to be made to facilitate the optimal development of metronomic chemotherapy.

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Introduction

Despite intensive research, many adults and children with certain cancers still cannot be cured with current treatments. Moreover, conventional chemotherapy and even targeted therapy impose side effects that limit dosing and hamper the efficacy of anticancer treatments. Thus, new targets as well as new strategies to fight cancer are essential to improve treatment.[1] Research on tumor angiogenesis was initiated by the pioneering work of Judah Folkman.[2] Over the past 40 years, angiogenesis has gained the status of a key driver in the local and distant growth of cancer.[3] Therefore, anti-angiogenic treatments for cancer have been developed and are currently being investigated in the clinic with promising results. Nevertheless, both intrinsic and acquired resistance to anti-angiogenic drugs are emerging as clinically relevant issues.[4,5] More surprisingly, two recent animal studies revealed that VEGF-targeted anti-angiogenic drugs, while inhibiting primary tumor growth, could promote tumor invasiveness and metastasis.[6,7] This unexpected deleterious effect could potentially explain why VEGF-targeted therapies often prolong cancer patient overall survival by only months and relapse occurs as a result of metastatic disease.[3,8]

An alternative to these angiogenesis inhibitors that is generating clinical interest is metronomic chemotherapy.[9,10] In 1991, Kerbel suggested that anti-cancer agents may be able to target tumor vasculature because many of the endothelial cells that compose the wall of tumor blood vessels are immature and constantly proliferating.[11] 10 years later, Klement *et al.*[12] and Browder *et al.*[13] published two provocative pioneering articles showing that mice bearing subcutaneous tumors could respond to frequent repeated low doses of chemotherapy, even when they displayed acquired drug resistance to the same agents given in a conventional way. Hanahan *et al.*[14] coined the term metronomic to describe this type of treatment. Metronomic chemotherapy was then defined as the chronic administration of chemotherapeutic agents at relatively low, minimally toxic doses, and with no prolonged drug-free breaks. It is thought this type of chemotherapy inhibits tumor growth primarily through anti-angiogenic mechanisms while significantly reducing undesirable toxic side-effects. Further *in vitro* and *in vivo* data strengthened the rationale for using metronomic chemotherapy in the clinic.[9,10,15] Subsequent clinical trials were undertaken in adults and in children with various tumor types using different drugs, drug combinations, doses and schedules of administration. In the past 5 years, favorable results have been obtained in patients who were untreated or had previously received conventional chemotherapy for the treatment of various types of cancer.[16–37] However, in some cases, metronomic chemotherapy failed to significantly improve patient survival.[38,39] Overall, as anticipated from preclinical studies,[12,13] metronomic chemotherapy can induce tumor stabilization or tumor responses in patients with cancer that were refractory to treatment or

Competing interests
The authors declare no competing interests.

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Key points

- Metronomic chemotherapy is based on the chronic administration of chemotherapeutic agents at relatively low, minimally toxic doses, and with no prolonged drug-free breaks
- Metronomic chemotherapy was originally developed to overcome drug resistance by shifting the therapeutic target from tumor cells to the tumor vasculature
- In the past decade, several pilot and phase II clinical studies have established the potential efficacy and low toxicity of metronomic chemotherapy in adult and childhood cancer patients
- Metronomic chemotherapy combined with conventional chemotherapy, radiotherapy and/or targeted therapy is an emerging anti-cancer strategy
- Recent findings suggest that metronomic chemotherapy may be a multi-targeted cancer therapy rather than a simple anti-angiogenic therapy
- In addition to inhibiting tumor angiogenesis, metronomic chemotherapy might also restore anticancer immune response and induce tumor dormancy

Table 1 | Metronomic chemotherapy in breast cancer patients

Patient population	Metronomic protocol	RR and CB (%)	Best responses
Metastatic disease previously treated with conventional chemotherapy[16]	Cyclophosphamide (oral, daily) Methotrexate (oral, twice weekly)	RR: 19 CB: 32	CR: 2/63 PR: 10/63 SD: 8/63 all*
Untreated elderly[20]	Letrozole alone (oral, daily)	RR: 72	CR: 23/57 PR: 18/57
	Letrozole (oral, daily) and Cyclophosphamide (oral, daily)	RR: 88	CR: 25/57 PR: 25/57
Untreated or previously treated with conventional chemotherapy[17]	Cyclophosphamide (oral, daily) Methotrexate (oral, twice weekly)	RR: 21 CB: 41.5	CR: 3/86 PR: 15/86 SD: 18/86 all*
	Cyclophosphamide (oral, daily) Methotrexate (oral, twice weekly) Thalidomide (oral, daily)	RR: 12 CB: 41.5	CR: 3/85 PR: 7/85 SD: 25/85 all*
HER2+ metastatic disease, previously treated with trastuzumab and conventional chemotherapy[21]	Cyclophosphamide (oral, daily) Methotrexate (oral, twice weekly) Trastuzumab (every 3 weeks)	RR: 18 CB: 45	CR: 0/22 PR: 4/22 SD: 10/22 with 6*
Previously treated with conventional chemotherapy[22]	Capecitabine (oral, 3×/day) Cyclophosphamide (oral, daily) Bevacizumab (every 2 weeks)	RR: 48 CB: 68	CR: 1/46 PR: 21/46 SD: 19/46 with 8*
Anthracycline- and taxane-refractory metastatic disease[23]	Cyclophosphamide (oral, daily) Methotrexate (oral, twice weekly) Bevacizumab (every 2 weeks)	RR: 32 CB: 64	CR: 0/22 PR: 7/22 SD: 7/22 all*
Metastatic disease either untreated or previously treated with conventional chemotherapy[18,19]	Cyclophosphamide (oral, daily) Dalteparin (s.c., daily) Methotrexate (oral, twice weekly) Prednisone (oral, daily)	RR: 17 CB: 24	CR: 1/41 PR: 6/41 SD: 3/41 all*
	Cyclophosphamide (oral, daily) Prednisone (oral, every 2 days) Epirubicin (i.v., weekly) 5-fluorouracil (i.v., weekly) Vincristine (i.v., weekly)	RR: 59 CB: 93	CR: 12/63 PR: 25/63 SD: 22/63

*For more than 6 months. Abbreviations: CB, clinical benefit; CR, complete response; i.v., intravenous; PR, partial response; RR, response rate; s.c., subcutaneous; SD, stable disease.

relapsed after conventional chemotherapy. Moreover the duration of clinical benefit obtained with metronomic chemotherapy can be longer than the benefit obtained with more traditional approaches.[40,41]

In this Review, we focus on the results of the latest clinical trials investigating the safety and efficacy of metronomic chemotherapy for the treatment of pediatric and adult cancer patients. The different mechanisms involved in the anti-angiogenic activity of metronomic chemotherapy are briefly described and novel putative mechanisms of action are discussed. Future directions and research efforts that remain to be made in order to facilitate the optimal development of metronomic chemotherapy in the clinic are also highlighted.

Clinical evaluation in adults

To date, the largest clinical trials investigating metronomic chemotherapy efficacy have been conducted in patients with advanced breast cancer (Table 1).[16–23] In eight of these clinical trials, which included almost 500 patients in total, metronomic chemotherapy alone[16–19] or in combination with letrozole,[20] trastuzumab[21] or bevacizumab,[22,23] was shown to be an effective approach. Furthermore it was associated with minimal toxic effects for either primary systemic or palliative treatment of advanced, metastatic and/or resistant breast cancer patients. The average response rates—complete response (CR) plus partial response (PR)—and overall clinical benefit (CR + PR + stable disease [SD] >6 months) of metronomic chemotherapy reported in these studies were 39% (range 12–88%) and 57% (range 24–93%), respectively. Interestingly, following metronomic chemotherapy with cyclophosphamide and methotrexate in combination with trastuzumab, a clinical benefit of 46% was reported for all patients, and 27% in the subgroup of patients that were already resistant to trastuzumab.[21] The results of this small, non-randomized clinical trial were in accordance with a pre-clinical study of metronomic cyclophosphamide in combination with trastuzumab.[42] These data suggest that this combination could possibly prevent breast cancer relapse by delaying and/or overcoming acquired resistance to trastuzumab. Finally, a large randomized phase III clinical trial comparing a combination of daily oral uracil and tegafur with conventional chemotherapy recently showed that metronomic chemotherapy was a promising postoperative adjuvant treatment for patients with high-risk breast cancer.[43]

Elsewhere, positive results were also reported with various metronomic chemotherapy regimens for patients with recurrent ovarian cancer,[24,25] hormone-resistant prostate cancer[26–29] advanced multiple myeloma,[30] recurrent non-Hodgkin's lymphoma,[31] recurrent malignant glioma and glioblastoma,[32–34] metastatic or locally advanced neuroendocrine carcinoma[35] and advanced cancer of various tumor types (Table 2).[36,37,44] These clinical studies, which included more than 500 patients in total, showed an average response rate to metronomic chemotherapy and overall clinical benefit of 33% (range 13–69%) and 61% (range 30–93%), respectively. In most cases, the mean duration of response could reach several months and responders experienced increased overall survival and progression-free survival (PFS). Considering that most of the study populations

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Table 2 | Metronomic chemotherapy in adult patients with various cancer types

Patient population	Metronomic protocol	RR and CB (%)	Best responses
Recurrent ovarian cancer, previously treated with conventional chemotherapy[24,25]	Cyclophosphamide (oral, daily) Bevacizumab (every 2 weeks)	RR: 44 CB: 67	CR: 2/9 PR: 2/9 SD: 2/9
	Cyclophosphamide (oral, daily) Bevacizumab (every 2 weeks)	RR: 24 CB: 56	CR: 0/70 PR: 17/70 SD: 44/70 with 22*
Hormone-refractory prostate cancer, previously treated by androgen deprivation[26,28,29]	Cyclophosphamide (oral, daily) Dexamethasone (oral, daily)	RR: 64 CB: 70	CR: 22/34 SD: 2/34
	Cyclophosphamide (oral, daily) Cyclophosphamide (oral, daily)	RR: 45	OR: 26/58
	Dexamethasone (oral, daily) Celecoxib (oral, twice daily)	RR: 32	OR: 9/28
Aggressive relapsed or refractory non-Hodgkin's lymphoma[31]	Cyclophosphamide (oral, daily) Celecoxib (oral, twice daily)	RR: 37.5 CB: 59	CR: 2/32 PR: 10/32 SD: 7/32
Progressive multiple myeloma, previously treated with conventional chemotherapy[30]	Cyclophosphamide (oral, daily) Prednisone (oral, daily)	RR: 69	OR: 29/42
Recurrent glioblastoma and malignant glioma, previously treated with radiotherapy and/or conventional chemotherapy[32–34,38]	Temozolomide (oral, daily)	RR: 16 CB: 58	CR: 0/12 PR: 2/12 SD: 5/12
	Temozolomide (oral, daily)	RR: 14 CB: 59	CR: 0/49 PR: 7/49 SD: 22/49
	Etoposide (oral, daily, 3 weeks) Bevacizumab (every 2 weeks)	RR: 22 CB: 90	CR: 3/59 PR: 10/59 SD: 40/59
	Etoposide (oral, daily, 3 weeks) alternating with Cyclophosphamide (oral, daily, 3 weeks) Thalidomide (oral, daily) Celecoxib (oral, twice daily)	RR: 10	CR: 0/47 PR: 1/47 MR: 4/47 SD: 26/47
Progressive, locally advanced or metastatic renal-cell carcinoma[39,55]	Cyclophosphamide (oral, daily) Celecoxib (oral, twice daily)	RR: 3 CB: 12.5	CR: 0/32 PR: 1/32 SD: 3/32 all*
	Capecitabine (oral, twice daily) Gemcitabine (i.v., weekly) Sorafenib (oral, twice daily)	RR: 50 CB: 93	PR: 20/40 SD: 17/40
Metastatic or locally advanced neuroendocrine carcinoma[35]	5-Fluorouracil (i.v., daily) Long-acting release octreotide (monthly)	RR: 24 CB: 93	CR: 0/29 PR: 7/29 SD: 20/29
Metastatic melanoma, previously treated with conventional chemotherapy[52]	Paclitaxel (i.v. for 3 days, weekly) Celecoxib (oral, twice daily)	RR: 5 CB: 35	CR: 0/20 PR: 1/20 SD: 7/20 with 3*
Advanced solid tumors of various types, previously treated with conventional chemotherapy[36,37,44]	Cyclophosphamide (oral, daily) Rofecoxib (oral, twice daily) Vinblastine (i.v., weekly)±minocycline	RR: 13 CB: 30	CR: 2/47 PR: 4/47 SD: 8/47 all*
	Capecitabine (oral, twice daily)	CB: 34	SD: 11/35 all*
	Celecoxib (oral, twice daily) Vinorelbine (oral, 3 times per week)	RR: 13 CB: 45	OR: 8/62 SD: 20/62 all*

*For more than 6 months. Abbreviations: CB, clinical benefit; CR, complete response; i.v., intravenous; MR, minor response; OR, objective response; PR, partial response; RR, response rate; SD, stable disease.

had a very poor prognosis and often included heavily pre-treated patients with progressive and/or recurrent disease, these results were quite promising.

It is interesting to note that when the study population was heterogeneous, treatment response varied according to the type of cancer. For instance, a three-drug to four-drug metronomic chemotherapy regimen, including daily low-dose cyclophosphamide, weekly vinblastine, daily rofecoxib plus or minus minocycline, was evaluated in 47 patients with advanced solid tumors of various types (Table 2).[36] Overall, the results of this phase II clinical trial were quite disappointing (objective response rate of 13% and clinical benefit of 30%). However, the study population was extremely heterogenous and patients seemed to respond quite differently according to the primary tumor type, highlighting the need to develop reliable tools for patient selection and stratification. Some small pilot and retrospective studies

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Table 3 | Metronomic chemotherapy in pediatric cancer patients

Patient population	Metronomic protocol	RR and CB (%)	Best responses
Refractory or relapsing tumors of various types[58]	Vinblastine (i.v., 3 times per week) Celecoxib (oral, daily)	RR: 0 CB: 17	CR: 0/17 PR: 0/17 SD: 9/17 with 3* PD: 8/17
	Cyclophosphamide (oral, daily) Celecoxib (oral, daily)	RR: 0 CB: 6	CR: 0/16 PR: 0/16 SD: 7/16 with 1* PD: 9/16
Refractory or relapsing tumors of various types[41]	Etoposide (oral, daily, 3 weeks) alternating with cyclophosphamide (oral, daily, 3 weeks) Thalidomide (oral, daily) Celecoxib (oral, daily)	RR: 15 CB: 50	CR: 0/20 PR: 3/20 SD: 10/20 with 7* PD: 8/20
Refractory, relapsing or "high risk of relapse" tumors of various types[40]	Etoposide (oral, daily, 3 weeks) alternating with temozolomide (oral, daily, 6 weeks) Celecoxib (oral, daily) Retinoic acid (oral, daily, 2 weeks)	RR: 32 CB: 77	CR: 3/22 PR: 4/22 SD: 11/22 with 10* PD: 4/22
Refractory, relapsing or "high risk of relapse" brain tumors (age <5 years)[57]	Alternating combination every 3 weeks: celecoxib (oral, daily) and retinoic acid (oral, daily) and either temozolomide (oral, daily), etoposide (oral, daily) or cyclophosphamide (oral, daily)	RR: 20 CB: 80	CR: 0/10 PR: 2/10 SD: 6/10 all* PD: 2/10
Refractory, relapsing or "high risk of relapse" tumors[59]	Celecoxib (oral, daily)+etoposide (oral, daily, 2 weeks) alternating with cyclophosphamide (oral, daily, 2 weeks)	RR: 0 CB: 35	CR: 0/17 PR: 0/17 SD: 12/17 with 6* PD: 5/17

*For more than 6 months. Abbreviations: CB, clinical benefit; CR: complete response; OR: objective response; PD, progressive disease; PR: partial response; RR, response rate; SD, stable disease.

have also reported encouraging results for metronomic chemotherapy in the palliative treatment of patients with pre-treated advanced cancers, such as colorectal and gastric cancer,[45] advanced and/or metastatic melanoma,[46,47] and vascular tumors.[48] A retrospective analysis of nine multicenter phase II trials involving more than 200 patients with various tumor types also revealed the clinical efficacy of metronomic chemotherapy combined with a Cox-2 inhibitor, transcription modulators and anti-inflammatory drugs.[49] Finally, a large randomized phase III clinical trial investigating the effectiveness of uracil and tegafur showed an increase in overall survival of lung cancer patients,[50] further highlighting the clinical efficacy of metronomic chemotherapy.

In contrast with these encouraging results, at least four clinical trials have reported limited activity of metronomic chemotherapy. In 32 patients with progressive, locally advanced or metastatic renal-cell carcinoma, the combination of continuous low-dose cyclophosphamide and celecoxib induced only one PR and three patients had SD for longer than 6 months, resulting in a clinical benefit of 12.5%.[39] Furthermore, a clinical trial of daily low-dose cyclophosphamide in combination with methotrexate twice weekly in 10 patients with recurrent glioblastoma reported a 6-month PFS of 0% and the trial was closed prematurely.[51] A phase II study also evaluated the efficacy of a four-drug metronomic chemotherapy regimen including daily low-dose etoposide, alternating with daily low-dose cyclophosphamide and in combination with thalidomide and celecoxib, in adults with recurrent malignant gliomas.[38] Of the 47 patients assessed, only one patient showed a PR and four showed minor responses (<50% decrease in tumor volume). Despite SD being observed in 59% of patients at their first scan, this metronomic chemotherapy regimen did not significantly improve overall survival in this heavily pre-treated study population. Nevertheless, when comparing responders (PR, minor response and SD) to non-responders, there was a significant difference in overall survival (33 versus 20 weeks, $P<0.05$) and PFS (21% versus 0% at 6 months, $P<0.0001$). Finally, a phase II pilot trial evaluating the efficacy of a combination of metronomic paclitaxel with celecoxib in 20 patients with metastatic melanoma reported a 5% response rate (no CR and one PR) and 35% clinical benefit (seven SD, including three for more than 6 months).[52]

Importantly, these relatively disappointing results obtained with metronomic chemotherapy were exclusively reported in malignancies that are poorly responsive to conventional chemotherapy and with a dismal prognosis.[38,39,51,52] For instance, even if first-line chemotherapy with temozolomide in combination with radiotherapy can significantly improve survival of malignant glioma patients,[53] tumors almost always recur and are ultimately fatal. Similarly, a review of over 80 phase II clinical trials for the treatment of renal-cell carcinoma, conducted between 1983 and 1993 and testing various single and combination chemotherapy regimens reported an overall response rate of only 6%,[54] which is comparable with the results reported for cyclophosphamide-based metronomic chemotherapy in this type of cancer.[39] Moreover, a recent phase II trial has reported very promising results in metastatic renal-cell carcinoma patients using a combination of metronomic capecitabine, sorafenib and gemcitabine, with a response rate of 50% and a clinical benefit of 92.5%.[55]

Clinical evaluation in children

Despite the considerable increase in the overall cure rate of pediatric cancer patients over the past 50 years, 25% of all children diagnosed with all forms of cancer will still die as a result of their disease. New therapeutic strategies such as metronomic chemotherapy may have the potential to improve survival and quality of life of pediatric cancer patients by reducing the burden of adverse effects. The number of clinical trials of metronomic chemotherapy in children is still quite limited but some of the results are very promising (Table 3).

In 2002, Sterba *et al.*[56] first reported encouraging responses to metronomic temozolomide in combination with radiotherapy in children with medulloblastoma and then developed a pilot study of a four-drug metronomic chemotherapy regimen in children with relapsed solid tumors of various types.[40] The COMBAT protocol (Combined Oral Maintenance Biodifferentiating and Anti-angiogenic Therapy) evaluated in this study was based on continuous celecoxib in combination with retinoic acid and alternation of metronomic etoposide and metronomic temozolomide. Another metronomic

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chemotherapy regimen, involving continuous oral thalidomide and celecoxib with alternating metronomic etoposide and cyclophosphamide, was evaluated in 20 children with recurrent or progressive cancer of various tumor types.[41] Eight patients completed the 6 months of treatment and five patients were free from progression 2 years after treatment initiation, with three patients experiencing partial response. More recently, Choi *et al.*[57] performed a retrospective analysis on 10 children with tumors of the central nervous system. Shortly after treatment with high-dose chemotherapy, these patients were treated with a five-drug metronomic chemotherapy regimen, including 21-day alternating cycles of etoposide, cyclophosphamide and temozolomide in combination with alternating celecoxib and retinoic acid. The clinical benefit reached 64%, 40% and 25% in these three studies, respectively.[40,41,57] Most importantly, Kieran *et al.*[41] and Choi *et al.*[57] reported residual tumors remaining stable in five patients for both studies even after cessation of metronomic treatment. Besides these promising results, some clinical studies performed on heterogeneous pediatric populations reported less encouraging data. For example, no objective response was observed when combining metronomic vinblastine and celecoxib, metronomic cyclophosphamide and celecoxib[58] or alternating metronomic cyclophosphamide, etoposide and celecoxib[59] in children with recurrent solid tumors. Nevertheless, a significant proportion of patients (13% and 41% in the first and second study, respectively) had durable SD (range 5–18 months).

Toxicity of metronomic chemotherapy

Despite the discrepancy of efficacy reported for metronomic chemotherapy based on the study population, drug combination and tumor type, the totality of clinical trials demonstrated that metronomic chemotherapy, alone or in combination, was well tolerated. High-grade toxic effects were either rare or not found. Most common toxic effects were grade 1 nausea and/or vomiting, grade 1 and 2 anemia, neutropenia, leucopoenia and lymphopenia as well as low-grade fatigue. Some toxic effects were also observed when metronomic chemotherapy was combined with other agents, such as bevacizumab.[24,25,34] In these studies, the combination of metronomic chemotherapy and bevacizumab did not seem to significantly increase the respective toxicity of each component of the treatment.[24,25,34] However, data are still limited and definitive conclusions cannot be drawn regarding the tolerance of these combinations. Overall, metronomic chemotherapy is associated with minimal toxicity and can provide significant clinical benefit and improve the quality of life of patients with advanced and/or relapsed cancer.

Nevertheless, some potential risks and concerns must be taken into account when using metronomic chemotherapy for an extended period of time, especially in children. First, as angiogenesis plays an important role in growth, special attention must be given to the development of young children receiving metronomic chemotherapy, although physiologic angiogenesis mechanisms may differ from tumor neo-angiogenesis. Second, prolonged metronomic chemotherapy may lead to high total cumulated doses of anticancer agents, which can be associated with secondary diseases. For instance, high cumulated dose of etoposide[60] or temozolomide[61] can lead to the development of secondary leukemia. Third, we recently reported occurrence of unusual subdural hematoma, potentially associated with metronomic chemotherapy, in a child with medulloblastoma.[62] Careful monitoring of children receiving metronomic chemotherapy will be required in any future studies.

New mechanisms of action

Metronomic chemotherapy is thought to exert its anticancer activity mainly by inhibiting tumor angiogenesis. Indeed, metronomic schedule of drug administration has been shown to considerably increase the anti-angiogenic properties of some chemotherapeutic drugs *in vitro*, such as cyclophosphamide and taxanes.[63] The anti-angiogenic activity of metronomic chemotherapy has been further demonstrated *in vivo* and several mechanisms of action have been identified. These include selective inhibition of proliferation and/or induction of apoptosis of activated endothelial cells, selective inhibition of endothelial cell migration, increase in the expression level of the endogenous angiogenesis inhibitor thrombospondin-1 and sustained decrease in levels and viability of bone marrow-derived endothelial progenitor cells. These anti-angiogenic mechanisms have been extensively reviewed[9,64–66] and will not be discussed here. Rather, we will focus on more recently discovered and new putative mechanisms. The classic theory regarding the mechanism of action of metronomic chemotherapy relies on the paradigm initially proposed by Folkman and Kerbel.[2,11] According to this paradigm, by targeting tumor endothelial cells, metronomic chemotherapy should be able to indirectly destroy drug-naive and drug-resistant cancer cells by induction of hypoxia and starvation of nutrients. This theory has been validated in several basic, preclinical and clinical research studies. However, additional mechanisms of action may be involved.

Activation of immunity

Growing evidence indicates that the innate and adaptive immune system has a key role in the development and the control of cancer. Indeed, Dunn *et al.*[67] have proposed that tumor escape from immuno-surveillance could be the seventh hallmark of cancer. Besides the well-characterized deleterious side effects of chemotherapy on the immune system, such as neutropenia and lymphopenia, various studies suggest that certain cytotoxic drugs, such as anthracyclines, taxanes and cyclophosphamide, display important immunostimulatory properties. These effects of chemotherapy on the immune system have been recently reviewed.[68] Among them, the effect on regulatory T cells (T_{REG}) seem to be quite relevant in the context of metronomic treatments. T_{REG} are $CD4^+$ $CD25^+$ lymphocytes, enriched in FoxP3, glucocorticoid-induced TNF receptor and cytotoxic T-lymphocyte-associated antigen-4 that can inhibit

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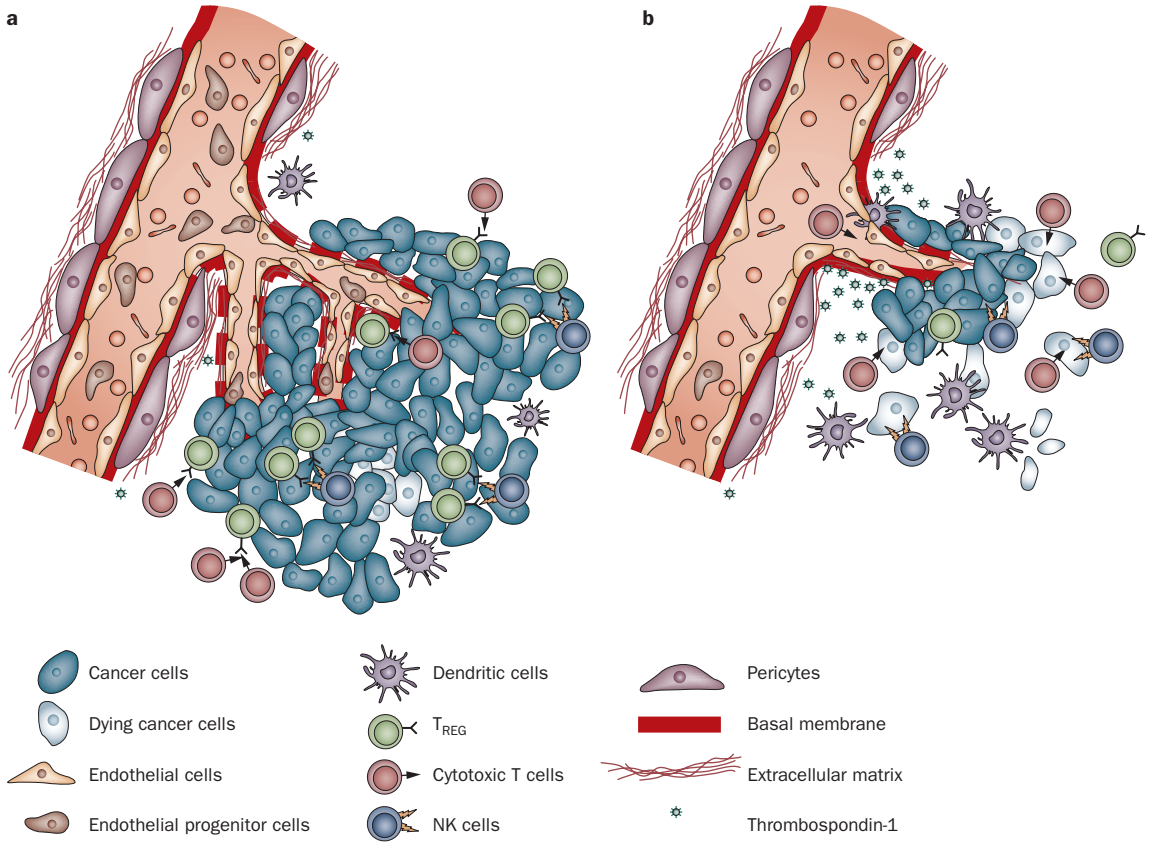

Figure 1 | Schematic model of a tumor and its microenvironment. Metronomic chemotherapy induces important anti-angiogenic effects (inhibition of endothelial cell proliferation, migration and morphogenesis, decrease in mobilization and viability of endothelial progenitor cells and increase in Thrombospondin-1 expression), resulting in a reduction of the tumor vasculature. In addition, metronomic chemotherapy also decreases the number and activity of regulatory T cells (T_{REG}) and may promote dendritic cell maturation, leading to (re)activation of an anticancer immune response, in part mediated by cytotoxic T cells and natural killer (NK) cells. The tumor and its surroundings **a** | before metronomic chemotherapy and **b** | after metronomic chemotherapy.

antigen-specific immune response both in a cytokine-dependent and cell contact-dependent manner.[69] T_{REG} can thus inhibit anti-tumor immune response by suppressing the activity of both tumor-specific ($CD8^+$ cytotoxic T lymphocytes and $CD4^+$ T helper cells) and tumor-unspecific effector cells (natural killer [NK] and NK T cells).[69] Interestingly, T_{REG} cells have been shown to be in increased proportions in a variety of human cancers. Increased frequency of T_{REG} cells can correlate with tumor progression and lack of treatment response.[70] Moreover, impairment of T_{REG} activity by either specific blockade or depletion can enhance immune response against tumor-associated antigens.[69]

Several studies performed in tumor-bearing animals have shown that low-dose cyclophosphamide can increase anti-tumor immune response by decreasing numbers and inhibiting the suppressive functions of T_{REG} cells but also by increasing both lymphocyte proliferation and memory T cells.[71–73] Low-dose metronomic temozolomide treatment also resulted in both depletion and inhibition of the immunosuppressive activity of T_{REG} in a rat model of glioma.[74] Moreover, Ghiringhelli *et al.*[75] recently demonstrated that metronomic cyclophosphamide reduced both the frequency of circulating T_{REG} cells and their immunosuppressive functions in advanced cancer patients. NK cell cytotoxic activity and T-cell-receptor-induced T cell proliferation were subsequently restored in these patients. It should be noted that this effect on T_{REG} cells was selective because no significant decrease in the number of other immune cell types was observed.[75] Also, this effect was specific for low-dose cyclophosphamide because treatment with higher doses resulted in a depletion of all lymphocyte subpopulations. Similarly, Generali *et al.*[76] recently showed that the combination of letrozole with metronomic cyclophosphamide induced a significant reduction in the number of T_{REG} in elderly (<70 years of age) breast cancer patients.

Besides these inhibitory effects on T_{REG} and subsequent activation of NK and T cell antitumor activity, an additional immunostimulatory effect of chemotherapy has been recently uncovered. Using a stable murine dendritic cell line expressing *YFP* under the control of the IL-1β promoter, Tanaka and coauthors investigated the capacity of 54 different chemotherapeutic drugs, belonging to four different classes (microtubule-targeting agents, topoisomerase inhibitors, alkylating agents and

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anti-metabolites), to induce dendritic cell maturation.[77] Interestingly, they found that some of these chemotherapeutic drugs—including vinblastine, paclitaxel and etoposide that can be used in metronomic chemotherapy regimens—could promote dendritic cell maturation at non-toxic concentrations.[77] The same group further validated the biological relevance of this immunostimulatory effect *in vivo* by showing that local injection of vinblastine at low doses could trigger the maturation of tumor-infiltrating dendritic cells and thus stimulate anti-tumor immune response.[78] The involvement of dendritic cell maturation in the activity of metronomic chemotherapy still needs to be further investigated and confirmed.

Emerging evidence suggests that some chemotherapeutic drugs commonly used in metronomic protocols may act not only by inhibiting tumor angiogenesis but also by restoring anticancer immune response (Figure 1). It is interesting to note that pre-clinical studies of metronomic chemotherapy often involved immunodeficient mice, potentially explaining why the effects of metronomic chemotherapy on the immune system could not be unveiled sooner. Elsewhere, metronomic chemotherapy has been shown to be effective in different immunodeficient mouse models, especially when combined with anti-angiogenic molecules.[12,79,80] This finding suggests that despite the growing body of evidence supporting the involvement of the immune system in the anticancer activity of metronomic chemotherapy, its role may not always be critical. Interestingly, even if some preclinical studies reported some long-term tumor responses,[12,13] most of these immunodeficient mice eventually relapsed.[9,81] In their detailed review article, Kerbel and Kamen suggested that these relapses indicated some forms of acquired resistance such as altered drug metabolism, selection of mutant tumor cells resistant to hypoxia or vascular remodelling into more mature vessels.[9] However, it is also possible that the lack of an efficient immune system could be involved in these late relapses as well.

Induction of tumor dormancy

Experimental and clinical evidence supports the existence and the crucial role of tumor dormancy both in repression of cancer progression and cancer relapse.[82,83] Tumor dormancy can thus be observed during the very early phase of cancer progression before the angiogenic switch occurs,[84] and also after completion of anticancer treatments during the remission phase where tumors can resume their growth from remaining residual disease many years later. For instance, in some extreme cases, circulating tumor cells can be found more than 20 years after mastectomy in patients who were without evidence of disease.[85] In addition, re-induction and maintenance of tumor dormancy constitute new desirable end points in cancer therapy.

Tumor dormancy occurs as a result of cell-cycle arrest or from a dynamic equilibrium state in which cell proliferation is balanced by apoptosis induction. It can take place at the primary site of cancer but also in metastatic sites, and it is usually divided into three different categories: angiogenic dormancy, cellular dormancy and immune-surveillance (Box 1). The different mechanisms involved in tumor dormancy have been recently reviewed in detail.[82] Since the initial work of Folkman and co-workers, it is known that avascular tumors can be maintained in a dormant state.[86,87] This kind of dormancy has been observed for instance in retinoblastoma.[88] Thus, by inhibiting tumor angiogenesis, metronomic chemotherapy may be able to induce and maintain angiogenic dormancy. Elsewhere, mechanisms involved in angiogenic dormancy may overlap with components of the immune system in the context of metronomic chemotherapy.

The immune system can participate in the control and eradication of cancer cells. Tumor-specific cytotoxic $CD8^+$ T lymphocytes, for instance, can induce tumor cell apoptosis. However, some residual cancer cells usually persist and they might be kept clinically dormant by the immune system.[89,90] Since an interruption of this dormancy state might be due to tumor cell escape from immune surveillance, the reinforcement of anticancer immunity by metronomic chemotherapy discussed above could constitute an alternative mechanism promoting tumor dormancy. A low level of persisting antigen is required for the maintenance of tumor dormancy and immunological T-cell memory.[91] Thus, one could postulate that the control, but not the eradication, of all cancer cells by this type of treatment may contribute to the reinforcement of the immuno-surveillance and the efficacy of metronomic chemotherapy in the long-term control of the disease. So far, there is no clear evidence that metronomic chemotherapy can directly induce cellular dormancy in cancer cells. Further studies are now essential to understand whether metronomic chemotherapy can promote tumor dormancy and to clarify the molecular and cellular mechanisms involved.

Box 1 | Metronomic chemotherapy and tumor dormancy

The mechanisms of tumor dormancy involve different interactions between cancer cells and their microenvironment, which can operate alone or in combination leading to angiogenic dormancy, cellular dormancy (G0–G1 arrest) and immuno-surveillance. Cellular dormancy may be driven by cancer cell senescence or quiescence.[82] While some markers of senescence such as p53 activation, p16 upregulation, expression of senescence-associated β-galactosidase (SA-β-gal) and development of senescence-associated heterochromatic foci[82,110] can be found in tumor cells exposed to chemotherapy,[111] quiescence may be more likely to explain cellular dormancy in the context of cancer. Indeed, quiescence is secondary to a G0–G1 arrest and Zetterberg and Larsson have reported that most of the adult cells exist in this state.[112] More importantly, quiescence can be reversed, a phenomenon observed in stem cells, and reversibility constitutes a major difference with other non-dividing cellular states, such as senescence, apoptosis, and terminal differentiation.[113] Coller *et al.*[113] showed that different quiescence states existed and could be induced by various initiating signals such as contact inhibition, loss of adhesion or growth-factor withdrawal. However, DNA microarray experiments revealed a common 'quiescence program' containing specific sets of genes controlling and ensuring the reversibility of the cell growth arrest.[113] To date, there is no evidence that metronomic chemotherapy can directly promote tumor dormancy and the potential mechanisms involved remain unknown. Nevertheless, it seems clear that the optimal metronomic chemotherapy regimen should at least aim at inducing and maintaining tumor dormancy, thus leading to long-term asymptomatic control of the disease.

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Box 2 | Metronomic chemotherapy and cancer cell evolution

Cancer cell evolution during chemotherapy is a complex multistep process involving numerous molecular pathways.[114] When exposed to chemotherapy, some cancer clones will survive because of the presence of resistance factors such as anti-apoptotic proteins or the multi-drug resistance phenotype.[115,116] Other cancer cell clones will be killed through apoptosis or non-apoptotic cell-death pathways. Models explaining this evolutionary process are based on Darwinian principles, which stipulate that evolution requires genetic and/or epigenetic changes to generate new phenotypes. In turn, the new individual phenotypes, through their interaction with their environment are selected upon their fitness, and therefore their capacity to favor cell proliferation and survival. In tumors, exposure to cancer treatment constitutes a major selection force that drives, at least partially, the capacity of every cancer clone generated within a tumor to survive and proliferate through genetic and epigenetic modifications. Cancer clones that can turn the barrier to proliferation into a driving force should have a major survival advantage.[117] When exposed to long-term continuous treatments such as metronomic chemotherapy, the majority of cancer clones may become resistant to chemotherapy and compete for survival and proliferation. Gatenby *et al.*[100] recently introduced the concept of adaptive therapy to explain the phenotypic divergence emerging through cancer cell evolution during chemotherapy. This innovative type of cancer treatment, which can be regarded as a gradually decreasing metronomic chemotherapy regimen, takes into account the complex interactions between resistant and non-resistant cancer clones. Adaptive therapy aims to maintain the equilibrium between these two populations to preserve a certain level of tumor sensitivity to treatment and induce lifetime-long control, rather than complete eradication, of the disease.[118] Elsewhere, emerging evidence of clonal heterogeneity within tumors adds an additional level of complexity to the biology of tumor development and paves the way for the development of new therapeutic strategies, integrating biological and ecological evolutionary perspectives.[119]

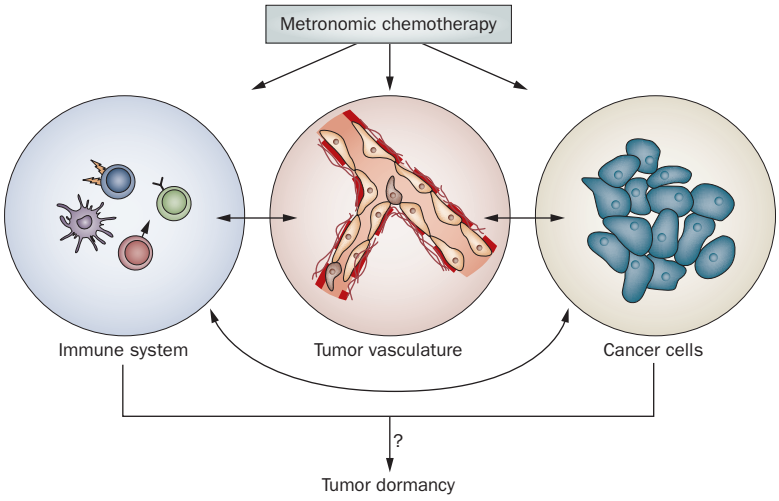

Figure 2 | Metronomic chemotherapy as a multi-targeted therapy. Metronomic chemotherapy exerts both direct and indirect effects on tumor cells and their microenvironment. It can inhibit tumor angiogenesis, stimulate anticancer immune response and may also be able to directly affect tumor cells through a theoretical drug-driven dependency/deprivation effect. Altogether, the direct effects of metronomic chemotherapy on the different compartments of the tumor microenvironment and the complex interactions between these compartments may lead to additional anticancer effects and potential (re)induction of tumor dormancy.

The 4D effect

Besides its effects on the tumor vasculature and the immune system, metronomic chemotherapy can probably also directly affect cancer cells. We have recently proposed a theoretical model of a new anticancer effect of metronomic chemotherapy based on the induction of chemotherapy-driven dependency of cancer cells followed by a forced drug deprivation occurring when treatment ends or during break periods. We named this phenomenon the drug-driven dependency/deprivation effect or 4D effect.[92] The rationale for this hypothesis is based on the principles of cancer cell evolution during long-term treatment (Box 2) and on recent clinical and pre-clinical evidence demonstrating the favorable effect of break introduction in long-term anticancer therapies. Seruga and Tannock recently suggested that intermittent, rather than continuous, androgen deprivation should become the standard therapy in prostate cancer.[93] In addition, preclinical evidence also suggests that introducing break periods in long-term treatments such as androgen deprivation in prostate cancer[94] or aromatase inhibition in breast cancer[95] could overcome or even reverse acquired drug resistance.

Elsewhere, *in vitro* data have shown that most microtubule-targeting agents (such as taxanes, *Vinca* alkaloid and epothilones) could induce dependency of tumor cell lines upon long-term drug exposure and induce cell death of these addicted cells upon drug withdrawal.[96–99] In the clinic, the three most efficacious metronomic protocols[40,41,57] that led to long-lasting responses or stabilization in children included four to five drugs and almost every drug was regularly stopped, constantly creating phases of long exposure to one or more agents and deprivation to others. It is thus plausible that creating phases of deprivation to one or more agents by introducing break periods in metronomic chemotherapy may increase treatment efficacy through this novel putative mechanism.

Overall, this theoretical model induces a major change in the paradigm in which the anticancer activity of metronomic chemotherapy does not rely on a direct effect on cancer cells but solely on endothelial cell-based anti-angiogenic effects. In our model, metronomic chemotherapy may target not only endothelial cells but also the immune system and potentially cancer cells and cancer stem cells through multiple direct and indirect effects, literally converting metronomic chemotherapy from an anti-angiogenic therapy to a multi-targeted therapy aimed at both tumor cells and their microenvironment (Figure 2).

Conclusions and future developments

Pre-clinical and clinical evidence supports metronomic chemotherapy as an efficient tool to fight certain types of cancer. However, the development of metronomic chemotherapy faces *terra incognita*. It seems very unlikely that a single metronomic regimen will have universal efficacy and the optimal combination regimens of metronomic chemotherapy remain to be determined for any given tumor type. Future preclinical and clinical studies will need to define the best agents to use according to tumor type, the number of agents to be incorporated, the doses of each agent to be used alone or in combination, and the timing of drug administration. Treatment duration and the best way to cease therapy also need to be

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optimized. That is, should metronomic chemotherapy be stopped abruptly or progressively as suggested by Gatenby's model of 'adaptive therapies'?[100]

Strategies are currently being developed in the clinic to combine metronomic chemotherapy with conventional chemotherapy given at the maximum tolerated dose, radiotherapy and/or targeted therapy, and such strategies are opening the way for a nearly infinite number of potential combinations. To help address these questions the development of reliable surrogate markers is mandatory. Kinetics and viability of circulating endothelial cells and progenitor endothelial cells could be potent predictive tools for patient stratification and treatment monitoring,[101–103] but the best methods of identification and measurement need to be determined. Elsewhere, one of the initial rationales for the development of antivascular therapies, and in particular metronomic chemotherapy, was based on the idea that their target (that is, vascular endothelial cells) is genetically stable and thus less likely to acquire drug resistance than cancer cells. However, this concept has been shown to be, at least in part, incorrect. Indeed, accumulating evidence suggests that tumor endothelial cells are significantly different from normal endothelial cells. They can harbor tumor-specific genetic abnormalities[104–106] and acquire drug resistance.[107–109] Therefore, in-depth pharmacogenetic and pharmacoproteomic studies on tumor endothelial cells are now needed to assess their sensitivity to metronomic chemotherapy and to help determine the most effective drug combination to use in the clinic. Finally, the new mechanisms of action of metronomic chemotherapy proposed here should also be considered when designing metronomic chemotherapy regimens and to monitor biological activity.

Review criteria

Information for this Review was compiled by searching the PubMed and MEDLINE databases for articles published before 16 February 2010, including early-release publications. Search terms included "metronomic chemotherapy", "low dose chemotherapy", "immunity and chemotherapy". Only articles published in English were considered and references were chosen based on the best clinical evidence. Full articles were obtained and references were checked for additional material when appropriate.

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